CLAIMS

1. A method of assaying a *Brassica* plant for imidazolinone herbicide tolerance conferred by the PM1 mutation of the *B. napus AHAS1* gene, the method comprising the steps of:

a) isolating genomic DNA from the plant;

b) selectively amplifying an *AHAS1* gene from the genomic DNA using an *AHAS1* forward primer and an *AHAS1* reverse primer in a first amplification step, thereby producing an *AHAS1* reaction mixture;

c) removing the *AHAS1* primers from the *AHAS1* reaction mixture to produce a purified *AHAS1* reaction mixture;

d) in a second amplification step, further amplifying a portion of the amplified *AHAS1* gene containing the site of the PM1 mutation, by combining the purified *AHAS1* reaction mixture with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer are nested within the *AHAS1* forward and reverse primers;

e) denaturing the product of the second amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions; and

f) detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded polynucleotide conformers in a substrate.

2. The method of claim 1, wherein the *AHAS1* forward primer has the sequence set forth in SEQ ID NO:9.

3. The method of claim 1, wherein the *AHAS1* reverse primer has the sequence set forth in SEQ ID NO:10.

4. The method of claim 1, wherein the PM1 forward primer has a sequence as set forth in SEQ ID NO:11.

5. The method of claim 1, wherein the PM1 reverse primer has a sequence as set forth in SEQ ID NO:12.

6. The method of claim 1, wherein step c includes incorporating a label into the amplified portion of the *AHAS1* gene.

7. The method of claim 6, wherein the label is selected from the group consisting of a radioactive label, a fluorescent label, a luminescent label, and a paramagmetic label.

8. The method of claim 1, wherein the substrate is selected from the group consisting of polyacrylamide, linear polyacrylamide, poly(N,N-dimethylacrylamide), hydroxyalkyl cellulose, polyoxyethylene, F127, agarose, diethylaminoethyl cellulose, sepharose, POP4, and POP6.

9. The method of claim 1, wherein the detection method is selected from the group consisting of electrophoresis and chromatography.

10. The method of claim 1, further comprising the step of detecting the presence or absence of PM2-mediated imidazolinone resistance in the plant.

11. A method for assaying a *Brassica* plant for imidazolinone herbicide tolerance conferred by the PM2 mutation of the *B. napus AHAS3* gene, the method comprising the steps of:

a) isolating genomic DNA from the plant;
b) selectively amplifying the *AHAS3* gene from the genomic DNA using an *AHAS3* forward primer and an *AHAS3* reverse primer in a first amplification step to produce an *AHAS3* reaction mixture;
c) removing the *AHAS3* primers from the *AHAS3* reaction mixture to produce a purified *AHAS3* reaction mixture;

d) in a second amplification step, further amplifying the amplified *AHAS3* gene, by combining a first aliquot of the purified *AHAS3* reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for a wild type allele of the PM2 region at position 1712 of the *AHAS3* gene as depicted in SEQ ID NOs:5 and 8;

e) in a third amplification step further amplifying the amplified *AHAS3* gene, by combining a second aliquot of the purified *AHAS3* reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for the PM2 mutation; and

f) analyzing the amplified first and second aliquots for the presence or absence of the PM2 mutation.

12. The method of claim 11, wherein wherein the *AHAS3* forward primer has the sequence set forth in SEQ ID NO:13.

13. The method of claim 11, wherein the *AHAS3* reverse primer has the sequence set forth in SEQ ID NO:14.

14. The method of claim 11, wherein the PM2 region forward primer has a sequence as set forth in SEQ ID NO:15.

15. The method of claim 11, wherein the PM2 region reverse primer has a sequence as set forth in SEQ ID NO:16.

16. The method of claim 11, wherein the wild type allele of the PM2 region at position 1712 has a sequence as set forth in SEQ ID NO:17.

17. The method of claim 11, wherein the primer selective for the PM2 mutation has a sequence as set forth in SEQ ID NO:18.

18. The method of claim 11, wherein steps c and d include incorporating a label into the amplified portion of the *AHAS3* gene.

19. The method of claim 18, wherein the label is selected from the group consisting of a radioactive label, a fluorescent label, a luminescent label, and a paramagmetic label.

20. The method of claim 11, wherein the analyzing step employs a method selected from the group consisting of electrophoresis and chromatography.

21. The method of claim 11, further comprising the steps of:

g) selectively amplifying an *AHAS1* gene from the genomic DNA using an *AHAS1* forward primer and an *AHAS1* reverse primer in a fourth amplification step;

h) removing the *AHAS1* primers from the product of step g);

i) in a fifth amplification step, further amplifying a portion of the amplified *AHAS1* gene containing the site of the PM1 mutation, by combining the product of step h) with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer are nested within the *AHAS1* forward and reverse primers;

j) denaturing the product of the fifth amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions; and

k) detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded conformer polynucleotides in a substrate.

22. An amplification primer selected from the group consisting of an oligonucleotide having a sequence as set forth in SEQ ID NO:9; an oligonucleotide having a sequence as set forth in SEQ ID NO:10; an

oligonucleotide having a sequence as set forth in SEQ ID NO:11; an oligonucleotide having a sequence as set forth in SEQ ID NO:12; an oligonucleotide having a sequence as set forth in SEQ ID NO:13; an oligonucleotide having a sequence as set forth in SEQ ID NO:14; an oligonucleotide having a sequence as set forth in SEQ ID NO:15; an oligonucleotide having a sequence as set forth in SEQ ID NO:16; an oligonucleotide having a sequence as set forth in SEQ ID NO:17; and an oligonucleotide having a sequence as set forth in SEQ ID NO:18.

23. A nucleic acid selected from the group consisting of a nucleic acid having a sequence as set forth from nucleotide 96 to nucleotide 2330 of SEQ ID NO:19; a nucleic acid having a sequence as set forth from nucleotide 1817 to nucleotide 2063 of SEQ ID NO:1; a nucleic acid having a sequence as set forth from nucleotide 1735 to nucleotide 1980 of SEQ ID NO:2; a nucleic acid having a sequence as set forth from nucleotide 1809 to nucleotide 2054 of SEQ ID NO:3; a nucleic acid having a sequence as set forth from nucleotide 1720 to nucleotide 1966 of SEQ ID NO:4; a nucleic acid having a sequence as set forth from nucleotide 64 to nucleotide 2310 of SEQ ID NO:20; a nucleic acid having a sequence as set forth from nucleotide 1383 to nucleotide 1770 of SEQ ID NO:5; a nucleic acid having a sequence as set forth from nucleotide 1518 to nucleotide 1905 of SEQ ID NO:6; a nucleic acid having a sequence as set forth from nucleotide 1352 to nucleotide 1739 of SEQ ID NO:7; a nucleic acid having a sequence as set forth from nucleotide 1308 to nucleotide 1695 of SEQ ID NO:8; a nucleic acid having a sequence as set forth from nucleotide 1560 to nucleotide 1770 of SEQ ID NO:5; a nucleic acid having a sequence as set forth from nucleotide 1695 to nucleotide 1905 of SEQ ID NO:6; a nucleic acid having a sequence as set forth from nucleotide 1529 to nucleotide 1739 of SEQ ID NO:7; and a nucleic acid having a sequence as set forth from nucleotide 1485 to nucleotide 1695 of SEQ ID NO:8.

24. A method of marker assisted breeding of plants of *Brassica* species using a PM1 mutation of the *B. napus AHAS1* gene as a marker, the method comprising the steps of:

a) isolating genomic DNA from a *Brassica* plant;

b) selectively amplifying an *AHAS1* gene from the genomic DNA using an *AHAS1* forward primer and an *AHAS1* reverse primer in a first amplification step, thereby producing an *AHAS1* reaction mixture;

c) removing the *AHAS1* primers from the *AHAS1* reaction mixture to produce a purified *AHAS1* reaction mixture;

d) in a second amplification step, further amplifying a portion of the amplified *AHAS1* gene containing the site of the PM1 mutation, by combining the purified *AHAS1* reaction mixture with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer are nested within the *AHAS1* forward and reverse primers;

e) denaturing the product of the second amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions;

f) detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded polynucleotide conformers in a substrate: and

g) selecting said plant as a parent for further breeding if the PM1 mutation is present.

25. A method of marker assisted breeding of plants of *Brassica* species using a PM2 mutation of the *B. napus AHAS3* gene as a marker, the method comprising the steps of:

a) isolating genomic DNA from the plant;

b) selectively amplifying the *AHAS3* gene from the genomic DNA using an *AHAS3* forward primer and an *AHAS3* reverse primer in a first amplification step to produce an *AHAS3* reaction mixture;

c) removing the *AHAS3* primers from the *AHAS3* reaction mixture to produce a purified *AHAS3* reaction mixture;

d) in a second amplification step, further amplifying the amplified *AHAS3* gene, by combining a first aliquot of the purified *AHAS3* reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for a wild type allele of the PM2 region at position 1712 of the *AHAS3* gene as depicted in SEQ ID NOs:5 and 8;

e) in a third amplification step further amplifying the amplified *AHAS3* gene, by combining a second aliquot of the purified *AHAS3* reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for the PM2 mutation;

f) analyzing the amplified first and second aliquots for the presence or absence of the PM2 mutation; and

g) selecting said plant as a parent for further breeding if the PM2 mutation is present.